

Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US04/043679

International filing date: 23 December 2004 (23.12.2004)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/532,088
Filing date: 23 December 2003 (23.12.2003)

Date of receipt at the International Bureau: 11 February 2005 (11.02.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)



World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

1280843

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

February 03, 2005

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: 60/532,088

FILING DATE: *December 23, 2003*

RELATED PCT APPLICATION NUMBER: *PCT/US04/43679*



Certified by

Under Secretary of Commerce
for Intellectual Property
and Director of the United States
Patent and Trademark Office



122303

17119 U.S. PTO

PTO/SB/16 (10-01)

Approved for use through 10/31/2002. OMB 0651-0032

U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53(c).

Express Mail Lab IN .

ER615368597US

INVENTOR(S)						
Given Name (first and middle [if any])		Family Name or Surname		Residence (City and either State or Foreign Country)		
Xing-Xiang		Li		Vienna, Virginia		
<input checked="" type="checkbox"/> Additional inventors are being named on the _____ (separately numbered sheets attached hereto)						
TITLE OF THE INVENTION (500 characters max)						
Directional labeling of signal and affinity moieties on microparticles						
Direct all correspondence to: CORRESPONDENCE ADDRESS						
<input type="checkbox"/> Customer Number		Type Customer Number here		Place Customer Number Bar Code Label here		
OR						
<input type="checkbox"/> Firm or Individual Name		Xing-Xiang Li				
Address		9023 Edgepark Rd				
Address						
City		Vienna	State	VA	ZIP	22182
Country		U.S.A	Telephone	(703)821-1612	Fax	(703)821-0299
ENCLOSED APPLICATION PARTS (check all that apply)						
<input checked="" type="checkbox"/> Specification Number of Pages		3		<input type="checkbox"/> CD(s), Number		
<input type="checkbox"/> Drawing(s) Number of Sheets				<input checked="" type="checkbox"/> Other (specify)		Self addressed card
<input type="checkbox"/> Application Data Sheet. See 37 CFR 1.76						
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT						
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27.						FILING FEE AMOUNT (\$)
<input type="checkbox"/> A check or money order is enclosed to cover the filing fees						
<input type="checkbox"/> The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number:						\$80.00
<input checked="" type="checkbox"/> Payment by credit card. Form PTO-2038 is attached.						
The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.						
<input type="checkbox"/> No.						
<input type="checkbox"/> Yes, the name of the U.S. Government agency and the Government contract number are: _____						

Respectfully submitted,

SIGNATURE

TYPED or PRINTED NAME

Xing-Xiang Li

TELEPHONE

(703)821-1612

Date

12-22-03

REGISTRATION NO.

(if appropriate)

Docket Number:

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, D.C. 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, D.C. 20231.

PROVISIONAL APPLICATION COVER SHEET
Additional Page

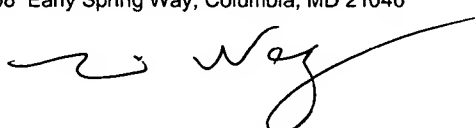
PTO/SB/16 (02-01)

Approved for use through 10/31/2002. OMB 0651-0032

U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Docket Number

INVENTOR(S)/APPLICANT(S)		
Given Name (first and middle [if any])	Family or Surname	Residence (City and either State or Foreign Country)
Tianxin	Wang	9768 Early Spring Way, Columbia, MD 21046 

Number 1 of 1

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

Directional Labeling of Signal and Affinity Moieties on Microparticles

This invention relates to a method or methods for labeling microparticles with signal generating moiety and analyte affinity moiety in a directional fashion. A variety of micro particles such as nano particles, micro spheres, micro beads and etc. can be employed in the invention. The purpose of this invention is to directionally label microparticles with both signal moiety and analyte affinity moiety on particle surface thereby minimizing potential interference of these two moieties for sensitive detection of an analyte. It may also and increase number of signal groups on the particle.

Microparticles may contain numerous functional chemical groups such as primary amine on their surface, which permits labeling of desired affinity groups for analyte capture. When both signal moiety (e.g., fluorescent compounds) and affinity moiety are present on particle surface or when the affinity group is on the surface while the signal molecules are encapsulated in the particles, microparticles may be used for detection of specific analytes. Microparticles are immobilized to a solid phase (e.g., magnetic particles) through the binding of specific analytes in the sample. After removal of unbound microparticles, the signal moiety /molecules are detected.

It is understood that microparticles that are applicable to this invention include, but are not limited to, microspheres, nanoparticles, liposome or the like, or other aggregates with appropriate surface functional groups.

Direct or indirect (e.g., through a carrier such as a polymer) labeling of signal compounds and affinity groups on to the particles can be applied. In both cases, however, the signal compounds may physically hinder the binding of affinity groups with analytes thereby reducing the binding efficiency of the affinity groups for the analytes.

In our invention, microparticles are directionally labeled with the affinity groups and signal compounds with the affinity groups present in the outermost layer while the signal compounds in a layer beneath the affinity group layer. An example is illustrated in Fig. 1. This layered or directional labeling method minimizes physical hindrance of the signal compounds with affinity binding thereby improving affinity binding efficiency. It may provide more signal molecules per particle for higher sensitivity.

The affinity group can be any chemical functionality with specific affinity for an analyte. These affinity groups include, but are not limited to deoxynucleic acid (DNA), antibody, antigen, and chelator. The signal compounds are those that can be detected using appropriate analytical methods. Preferably, but not necessarily, the signal compounds are those that produce fluorescence, chemiluminescence or electro-chemiluminescence. Examples of these signal compounds include fluorescent dyes, acridinium and its derivatives, and rare earth elements that produce fluorescence or electrochemiluminescence.

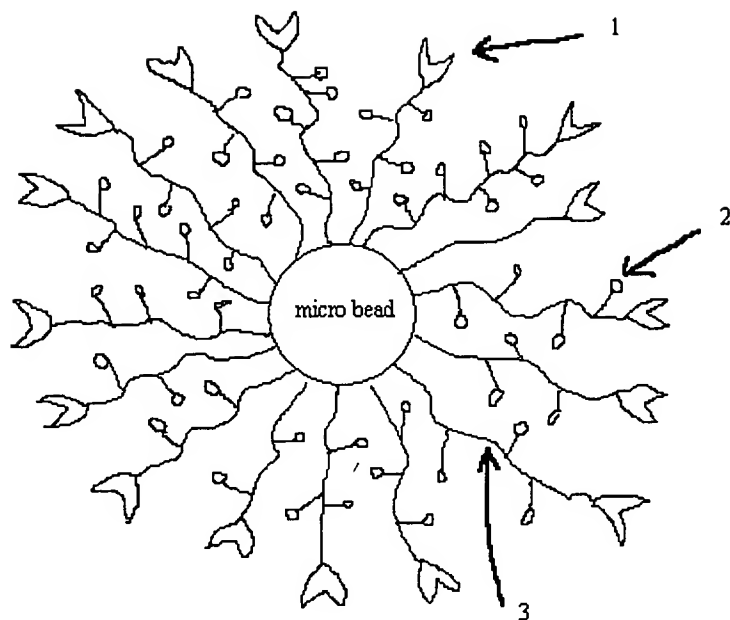


Fig 1 Layered amplifying micro sphere, 1: affinity group; 2: detecting tag (signal group) ; 3: polymer backbone

Directional labeling uses a polymer or polymers as the carrier to which both signal compounds and affinity groups can be labeled. Linear polymers (e.g., poly lysine, poly acrylic acid or modified nucleic acids) or highly branched macromolecules (such as dendrimers) can be used as the polymer backbone. They could be either natural or synthetic. One end of the polymer is attached to the microsphere whereas another end is linked to one or more affinity group; the signal group such as the fluorescent or chemoilluminant groups are normally conjugated to the polymer backbone or side chain in between the two termini. The linker between the signal groups and the polymer can also contain one or more cleavable bonds that can be cleaved using certain chemical (such as by adding a strong acid) or physical means (such as UV irradiation).

Preferably, but not necessarily, linkage between the polymers and particles and between polymer and affinity or signal groups involves covalent bonds. In certain situation, noncovalent linkage may provide a convenient alternative. For example, affinity group may be linked to the polymer via biotin-avidin binding, as illustrated in Figure 2. In this case, microparticles, which are labeled with biotin or avidin, are not analyte specific. Analyte-specific affinity group such as antibody is labeled with avidin or biotin and then bound to microparticles labeled with biotin or avidin, respectively.

Alternatively, the signal group and the affinity group can be coupled together to form a complex and the complex is coupled to the polymer or the surface of the micro particles without using any polymer.

In order to ensure that the affinity groups are located on the outermost surface of the microparticles, which provides faster and more efficient binding to the analyte, an hydrophilic group such as a PEG fragment can be attached to a position near the outer terminus of the polymer.

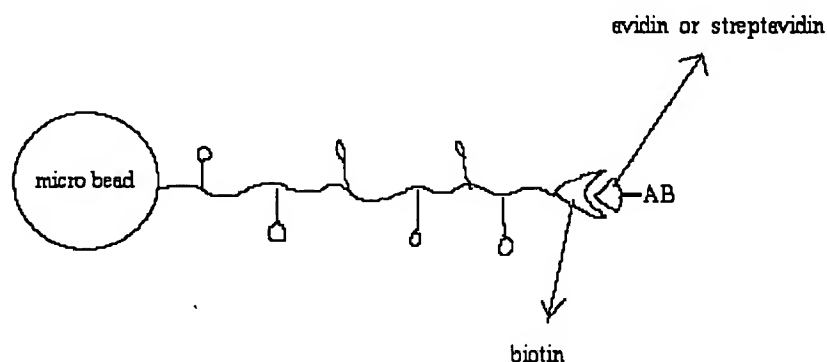


Fig 2. A universal microsphere frame for the layered amplifying micro particle, AB is antibody

The above design in fig 2. illustrates a versatile frame for this layered amplifying micro particle, using a micro particle having a self-assembly linker to couple the polymer with the affinity group (in the example here the linker is a biotin-streptavidin complex, the affinity group is a antibody AB), only different *affinity group - self-assembly linker* is need to be made for different application targeting different analytes.

It is within the scope of this invention that the affinity group is linked to microparticles through the signal group itself, as illustrated in Figure 3A. This method of labeling also gives rise to directional labeling. It is also within the scope of this invention that both the affinity group and signal group are directly labeled onto microparticles, but the linker for affinity group is sufficiently longer than that for signal group as shown in Figure 3B. The figure is a schematic view, the single S and A represents multiple S and A groups on the sphere MP.

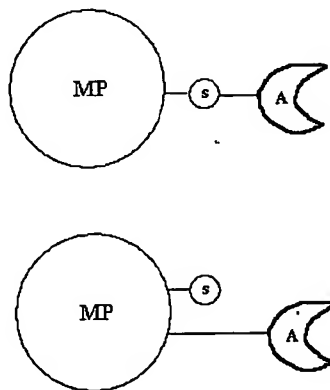


Figure 3A and 3B. (MP: microparticle; S: signal molecules; A: affinity groups)